

EECS C145B/ BioE C165

Lecture 17:

Optical imaging: bioluminescence, fluorescence,
laser Doppler and infrared

Jonathan S. Maltz

Fluorescence and bioluminescence material courtesy:

Arion Chatziioannou, Crump Institute of Molecular Imaging, UCLA

Guido Zavattini, Department of Bioengineering, UC Davis

Xenogen, Inc.

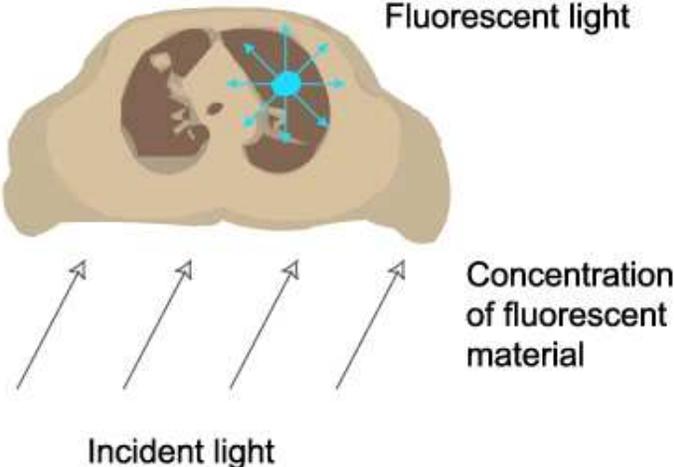
Infrared imaging material source:

IEEE Engineering in Medicine and Biology Magazine,

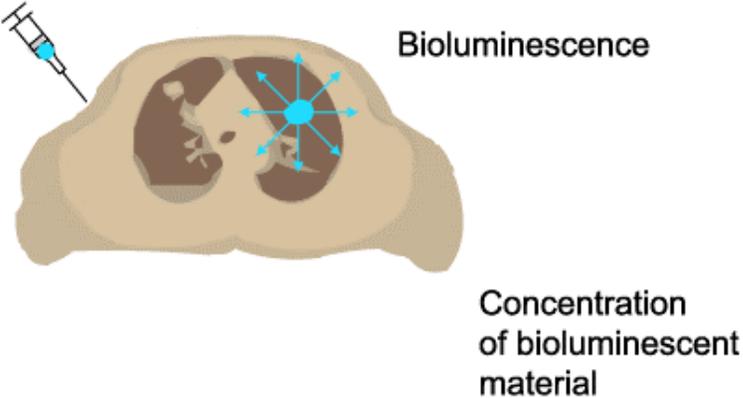
Vol. 21 No 6. November/December 2002

Contrast Mechanisms in Medical Imaging For 'Transparent' Animals Only

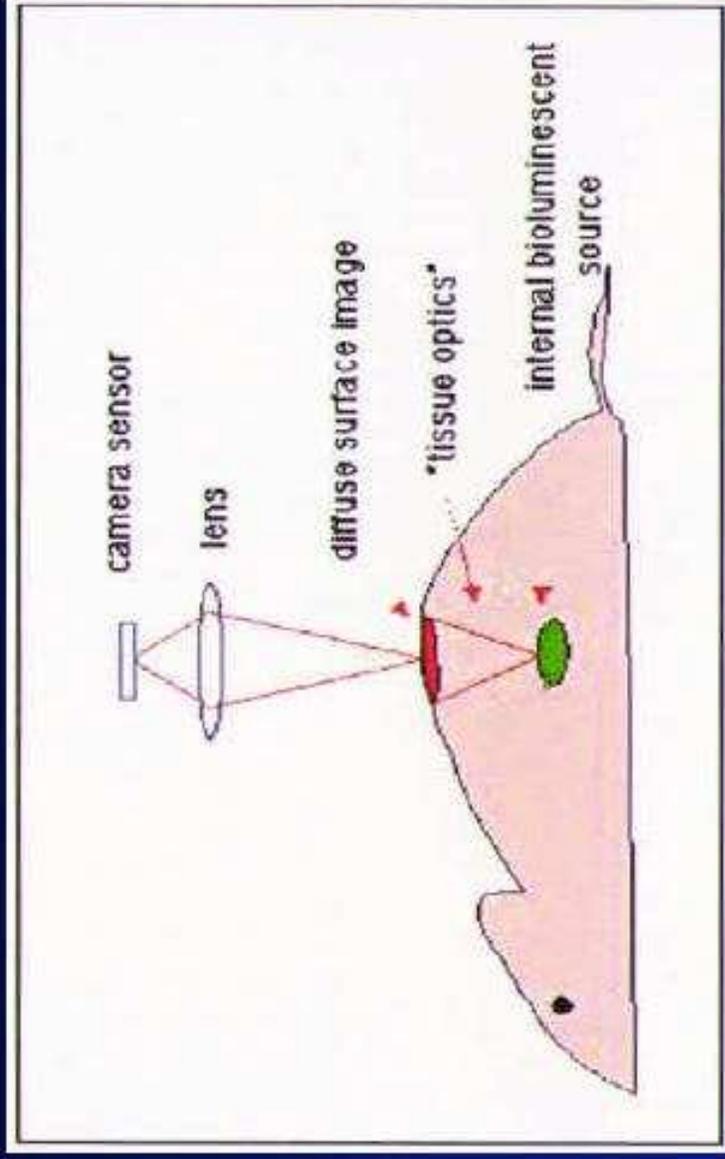
Optical



Bioluminescent substrate

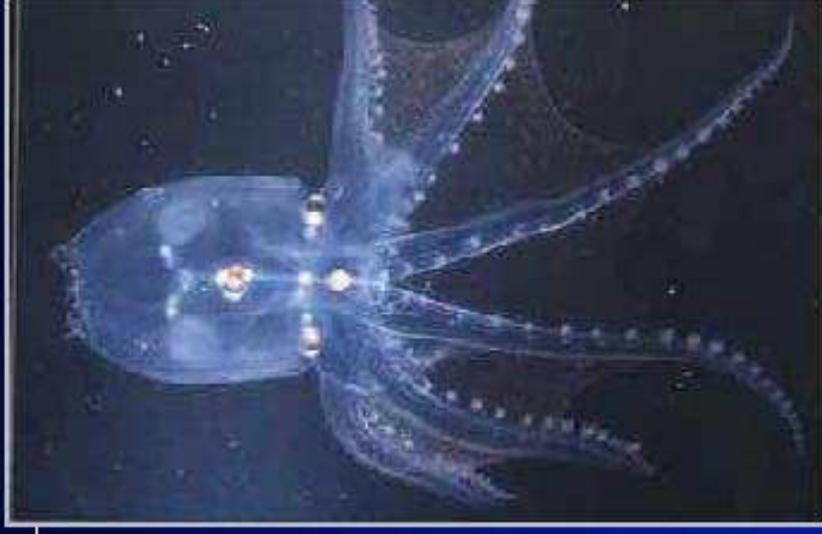


Optical Imaging



Tissue transparency

- Tissues can be transparent
- Retina cannot
- Stomach contents are not
- Limited to non-mammalian organisms, or special tissues



Light transport in tissue

- live tissue is relatively translucent, especially in the red band
- red (650nm): $\mu_a = 0.25$; $\mu_s' = 15$ (cm^{-1})
- orange (590nm): $\mu_a = 1.0$; $\mu_s' = 17$ (cm^{-1})
- green (550nm): $\mu_a = 5.0$; $\mu_s' = 20$ (cm^{-1})

μ_a = absorption coeff.; μ_s' = reduced scattering coeff.

At 1 cm depth red is attenuated factor ~ 100
green is attenuated a factor $\sim 10^{10}$.

Why bother?

- **Simple, easy to perform experiments**
- **Low cost instrumentation / probe development**
- **No ionizing radiation involved**
- **Carry-over of fluorescence/bioluminescence experiments in cell cultures**
- **Signal can be switched on – off**
- **No tomographic information**
- **Limited quantitation**



In vivo Bioluminescence Imaging

Luciferase (enzyme)

+

Luciferin (substrate)

+

energy

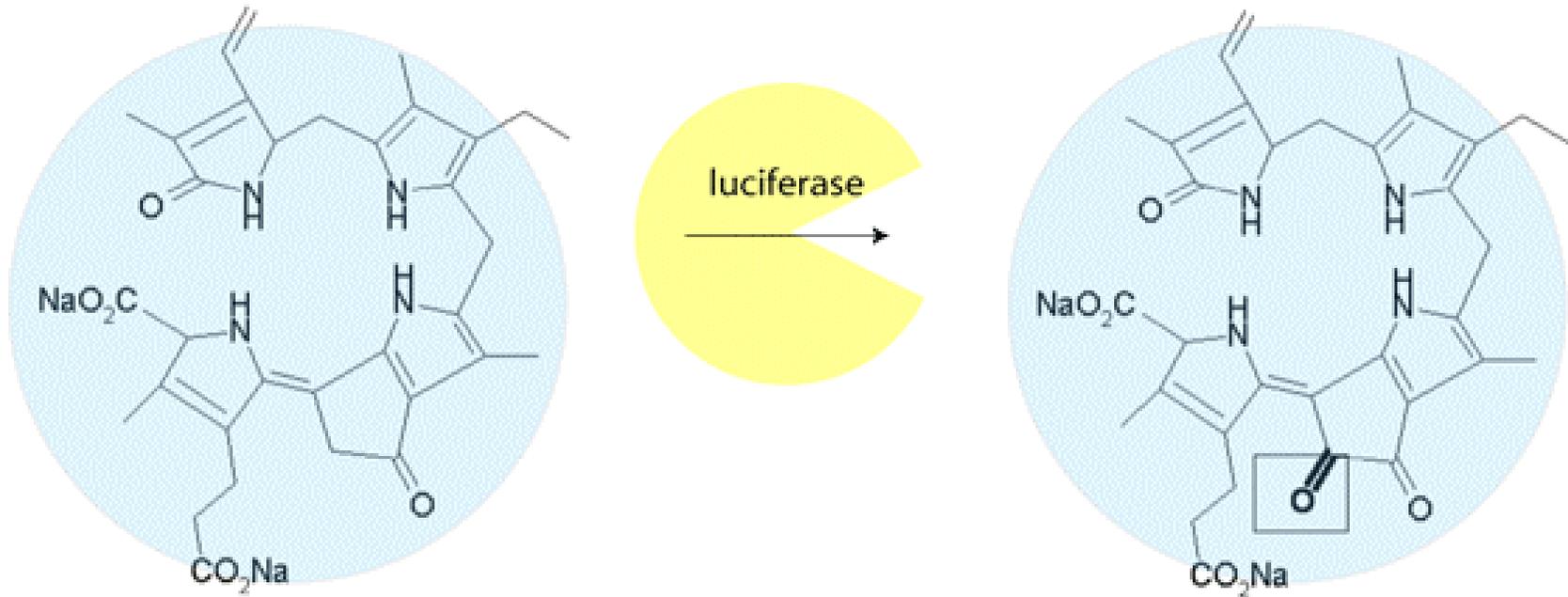


Light



Contrast Activation Chemistry

Bioluminescence

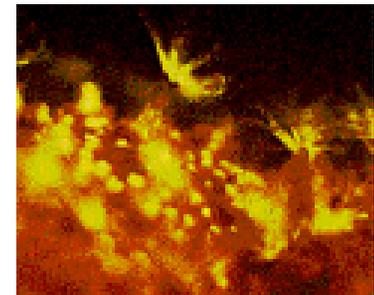


Luciferin/coelenterazine

Luciferase oxidation product

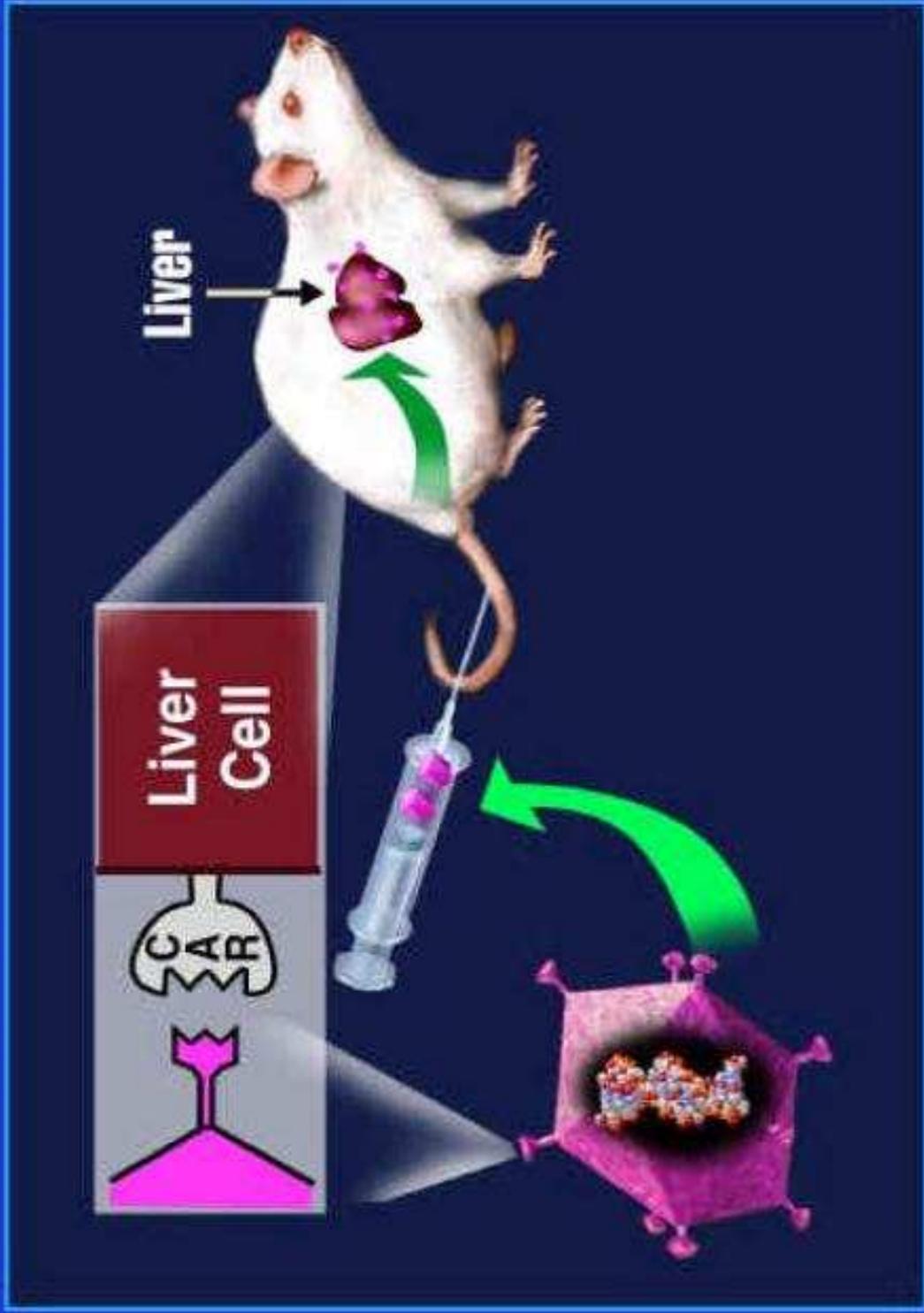


Firefly

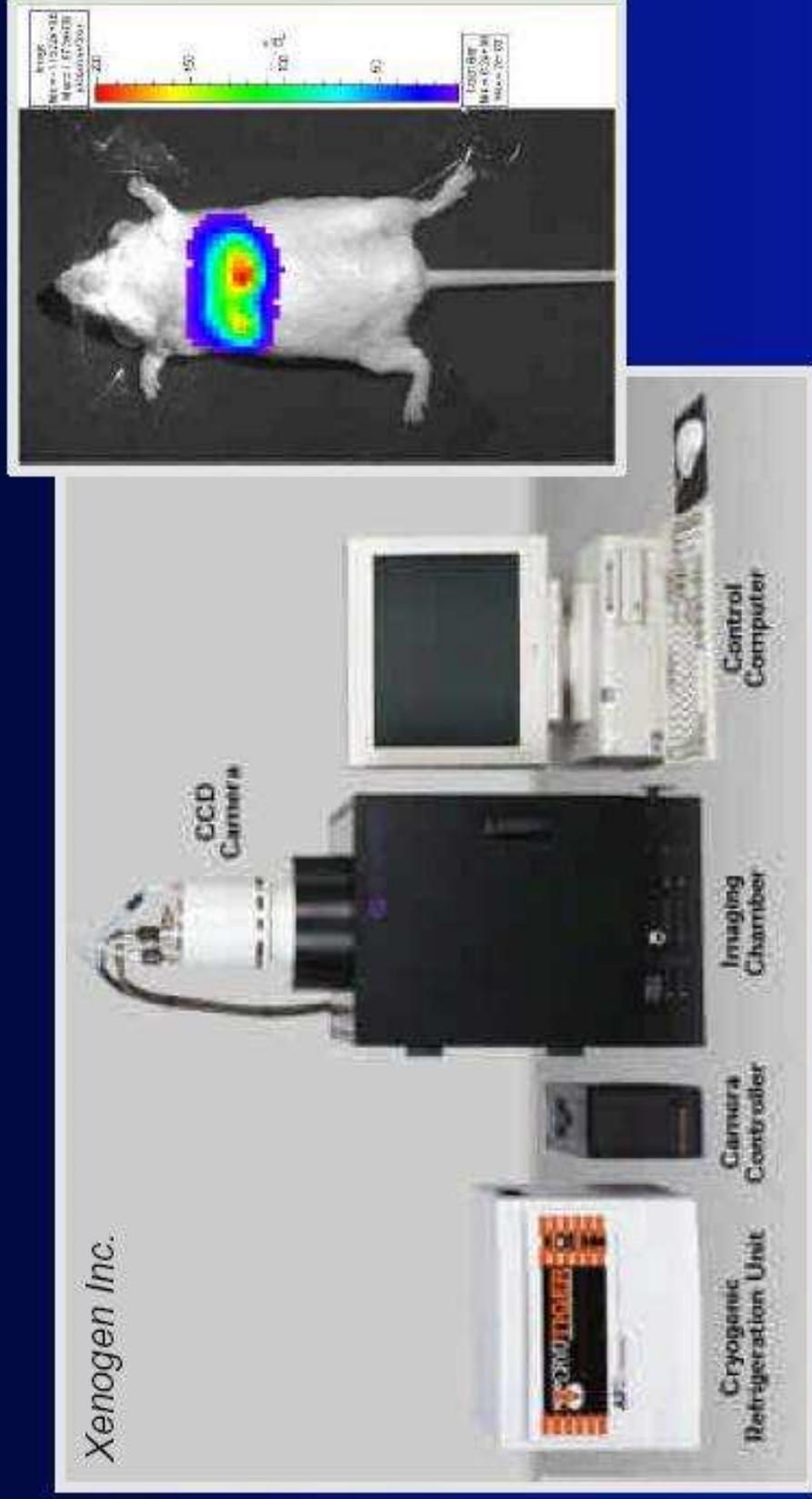


Sea pansy

Adenoviral Mediated Gene Delivery

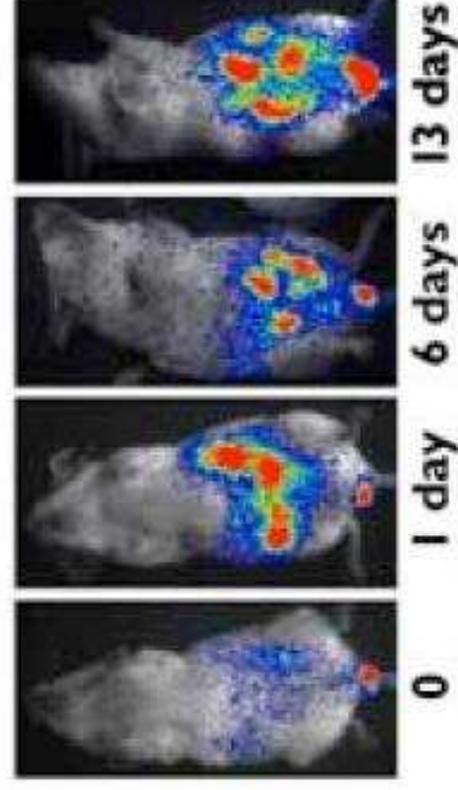
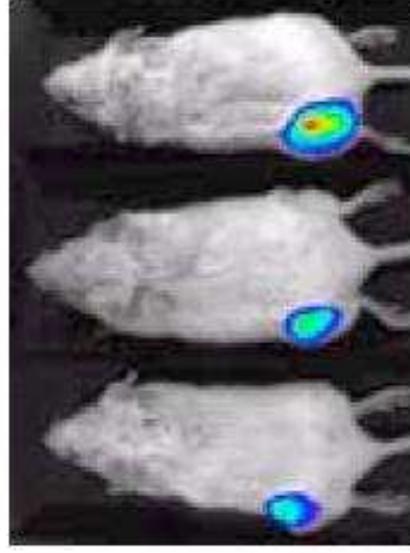


Optical Bioluminescence system



In-vivo bioluminescence imaging

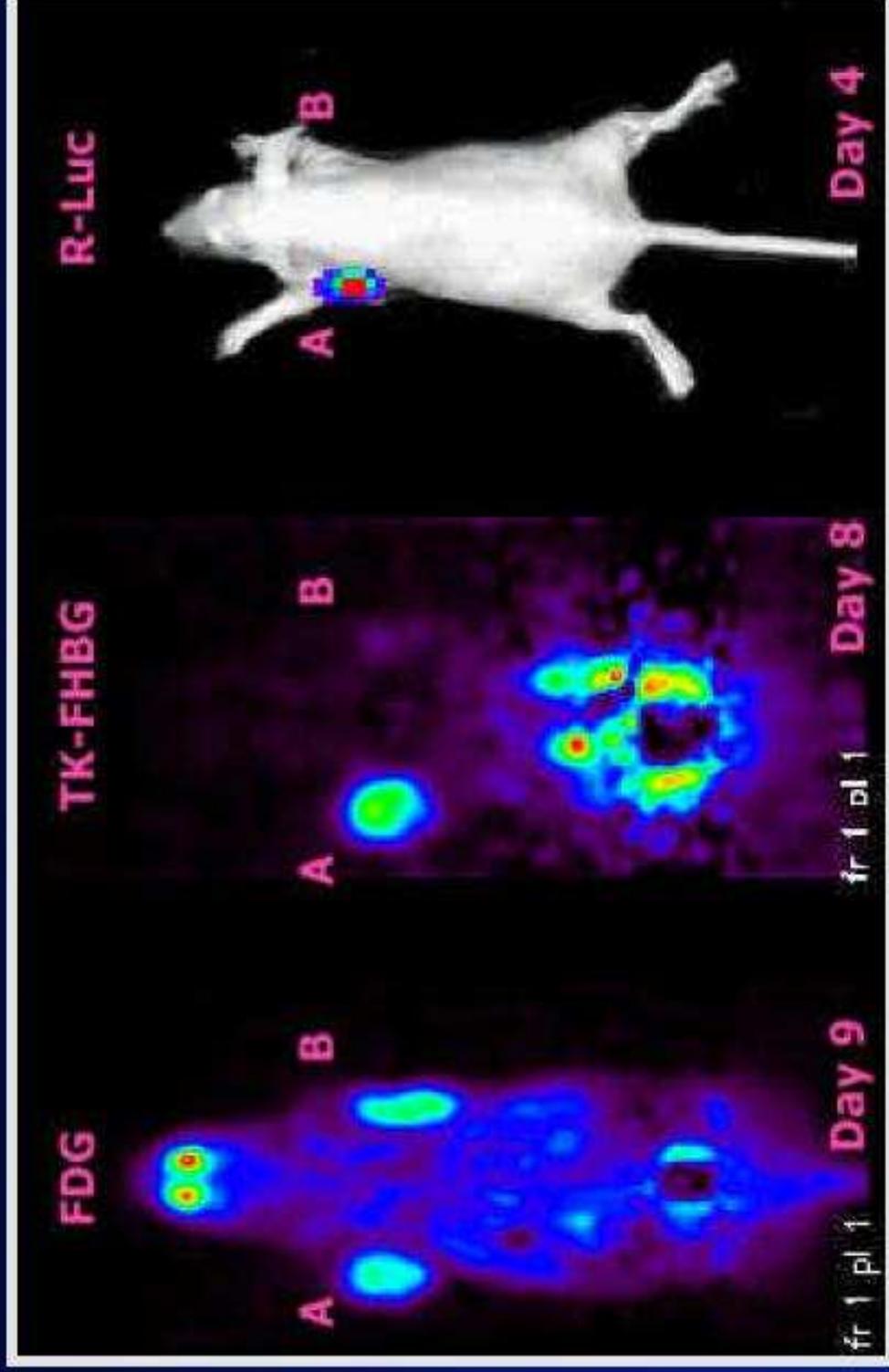
Uses an enzyme called luciferase



Tracking progress of disease.

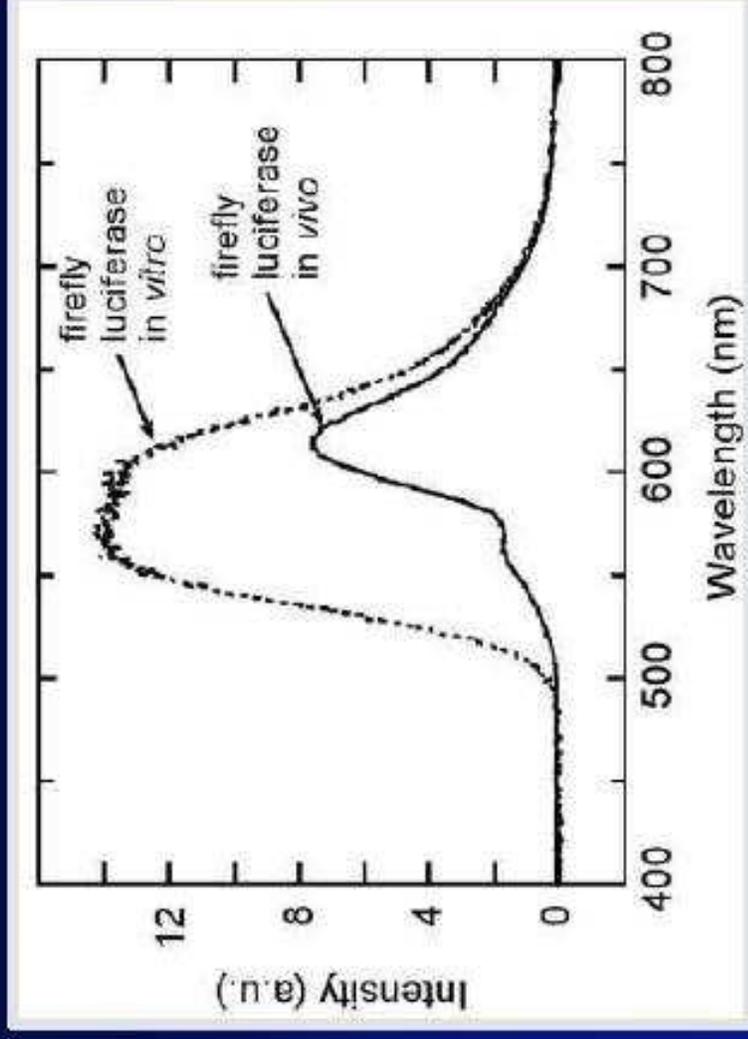
Tracking progress of tumor

Multimodality Biology



A- sr39-TK-RLuc fusion tumor, expressing both luciferase and +tk
B- N2a (control) tumor

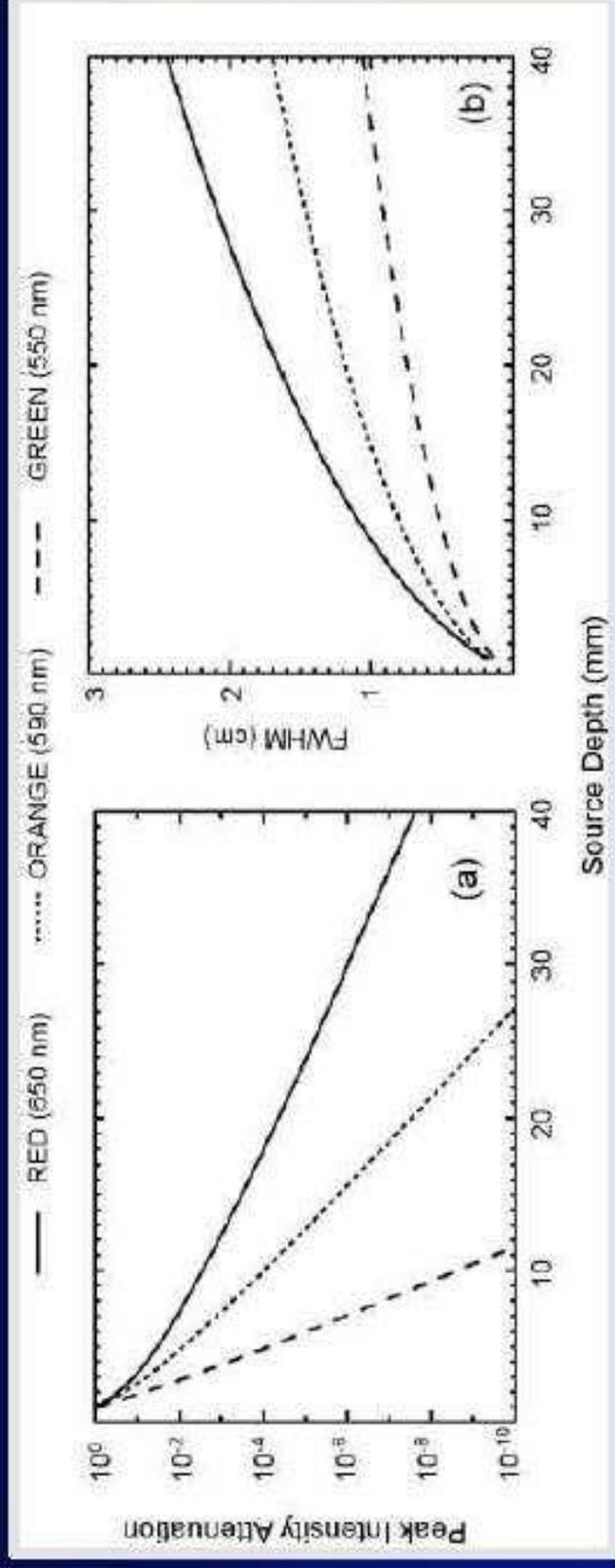
Luciferase spectrum



- Dramatic absorption of shorter wavelengths



Optical Imaging

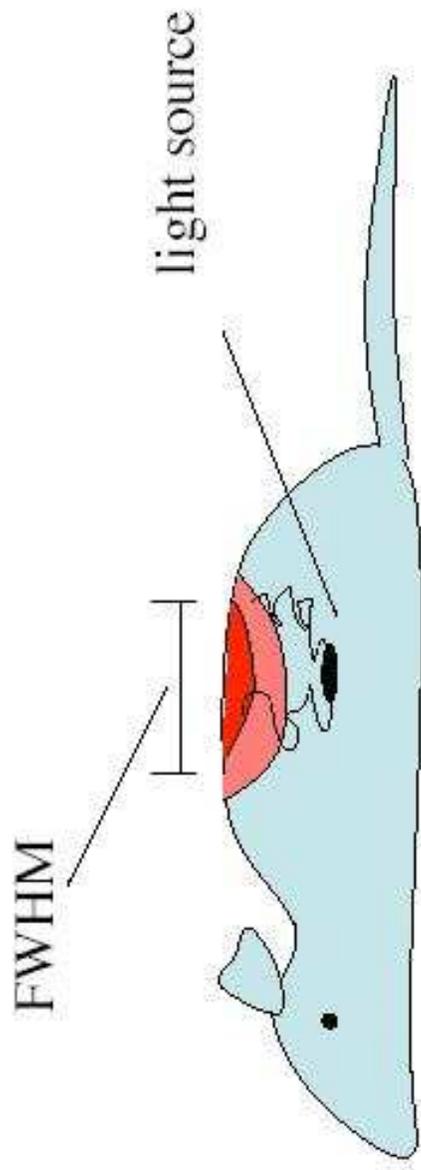


Light absorption

Spatial resolution



Light spot



@ 1 cm depth, FWHM = 1 cm for red light
FWHM = 0.7 cm for orange light
FWHM = 0.4 cm for green light

Fluorescence imaging

- Steady state light source: use filters to distinguish excitation light from fluorescence light
- With a pulsed light source and a gated readout one can measure the fluorescence decay time.
- Intensity modulated source also allows the measurement of the fluorescence decay time.

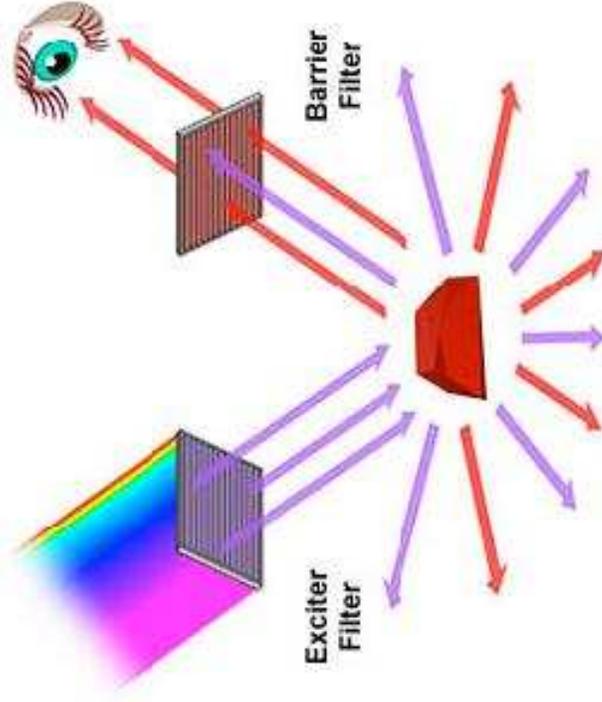
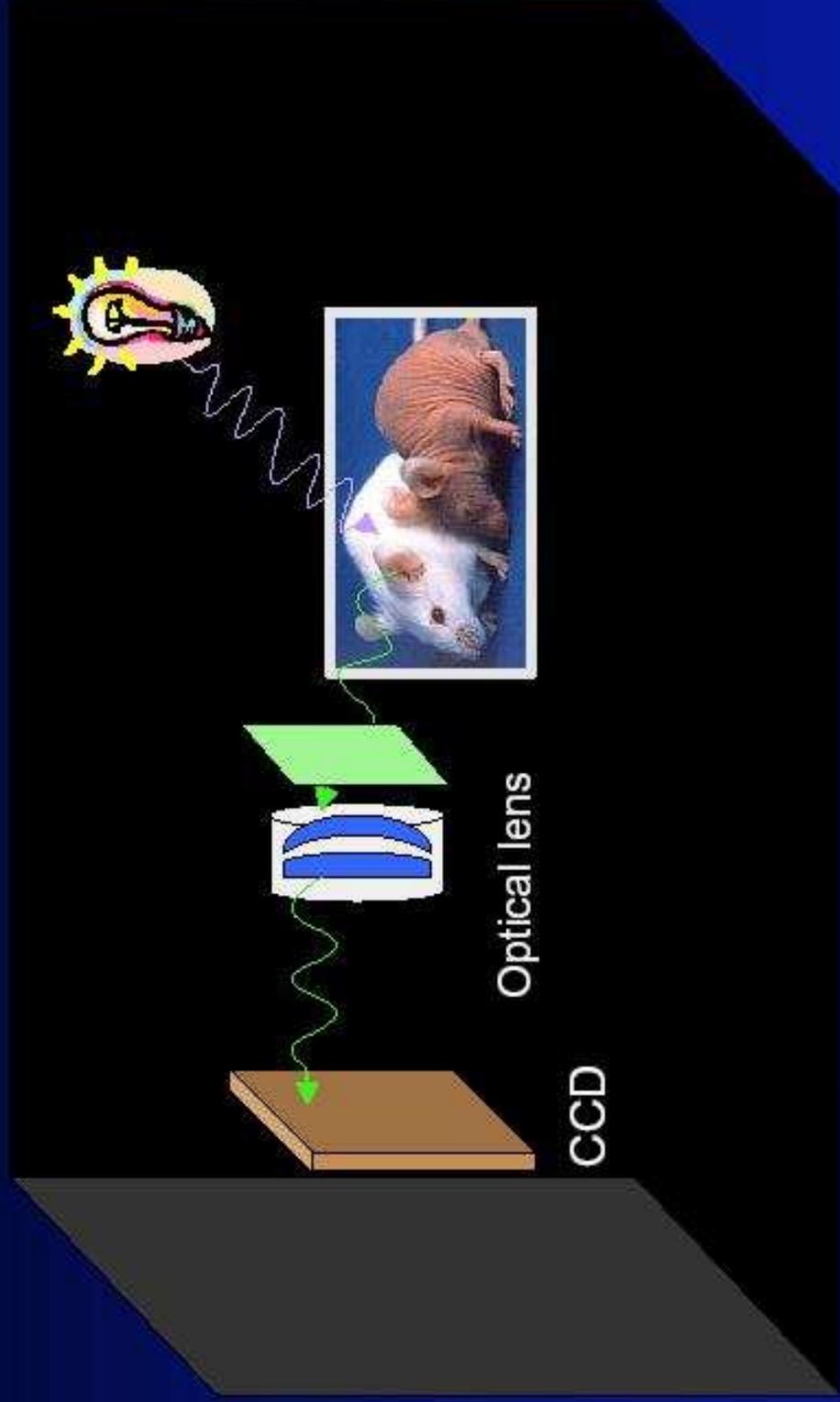


Figure 3

Fluorescence Imaging



In vivo GFP Imaging



Crump Institute for Molecular Imaging

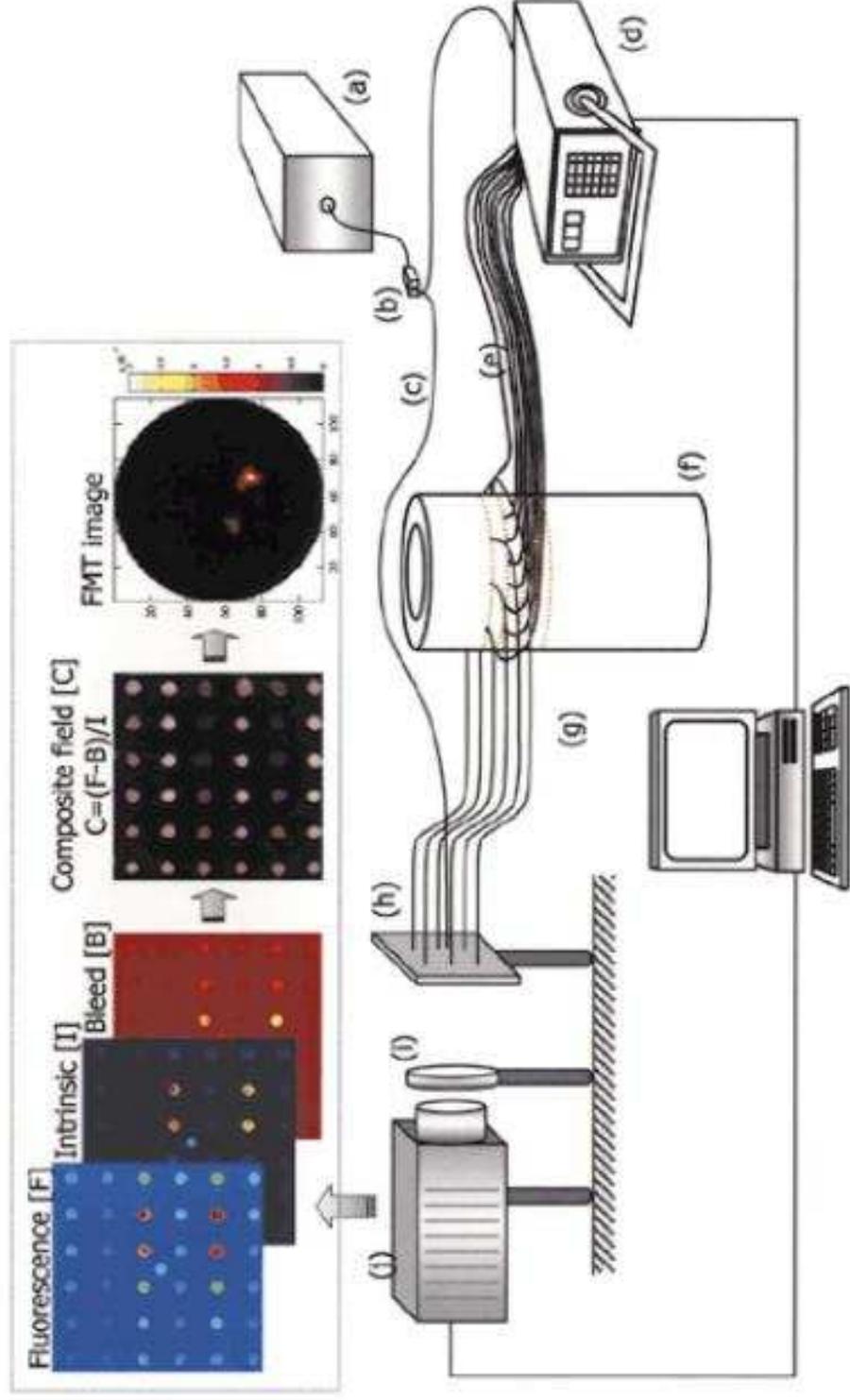


***In-vivo* fluorescence imaging**

- Excite fluorescence molecules within the small animal by illuminating from outside. Reconstruct a 3D image of the fluorophore distribution.
- **Advantages**
- Non radioactive (?)
- No decay involved. Molecule can always be reexcited. Follow long term processes.
- Potentially one can make multiple color images and follow simultaneously several drugs and/or processes.

Fluorescence tomography

V. Ntziachristos, R. Weissleder



Department of Biomedical Engineering, UC Davis

Fluorescence tomography 2

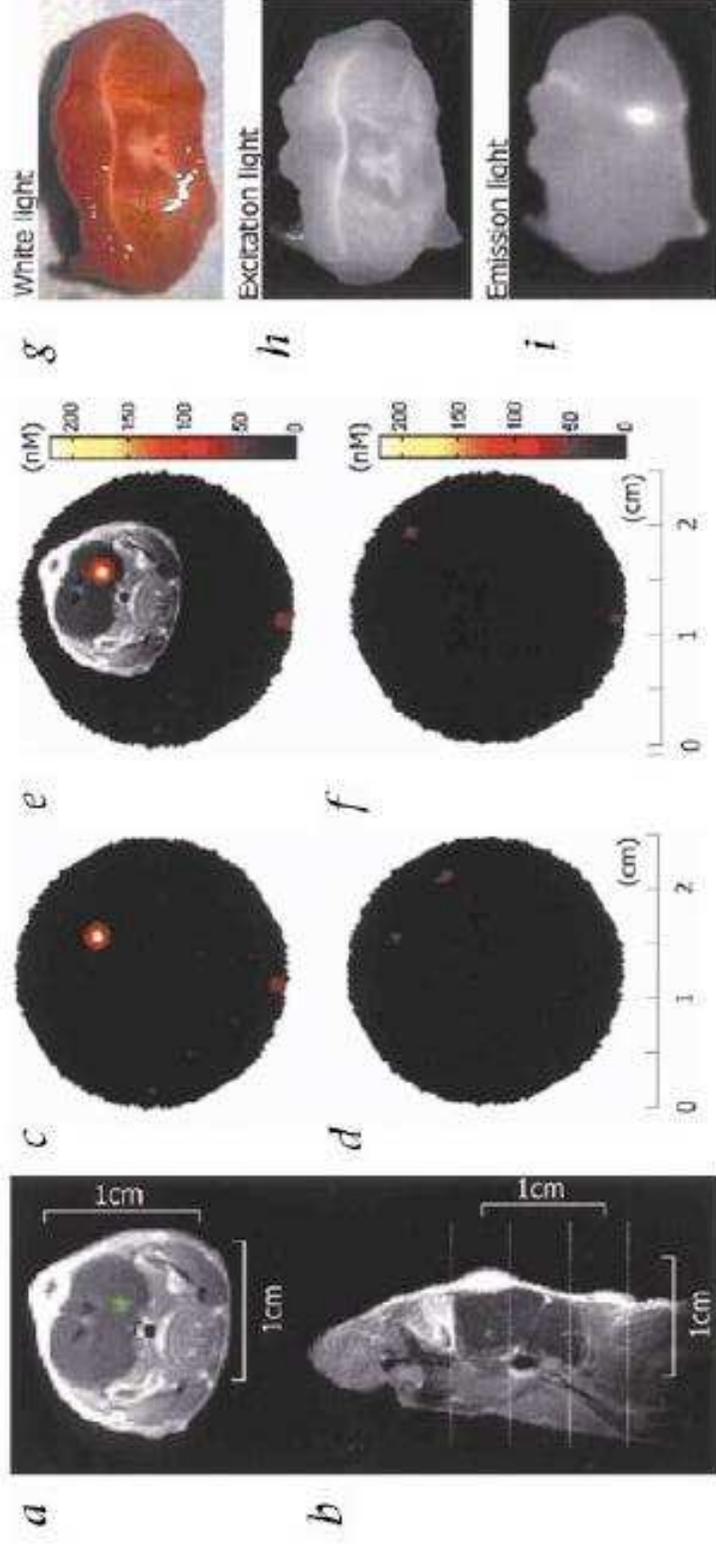
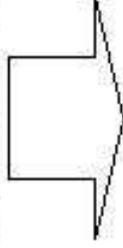


Figure 3: *In vivo* FMT of cathepsin B expression levels in 9L gliosarcomas stereotactically implanted into unilateral brain hemispheres of nude mice.

Try to introduce more information on depth of emission.
Try to measure simultaneously more colors.

- Spot size on surface of skin depends on depth of source.
- Spectrum of light exiting the mouse **also** depends on depth.

In the visible band **short wavelengths** are absorbed more than **long wavelengths**. Spectrum gets deformed as depth increases.



Measure both spectrum and image.
Need an Imaging Spectrograph

Department of Biomedical Engineering, UC Davis

Simultaneous colour and position measurement.

ImSpector + CCD

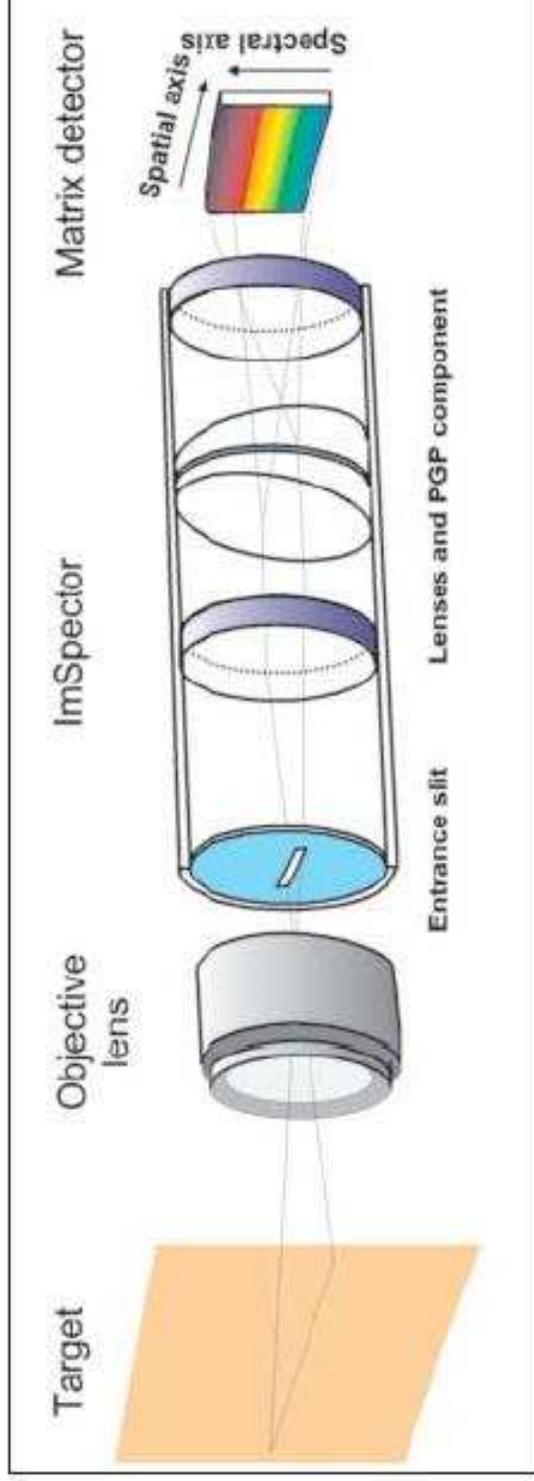


Figure 1. Operating principle of ImSpector



Black box assembly

Slit of ImSpector is verticle



Optical bench 120 cm x 60 cm.
Light tight box 60 cm high.

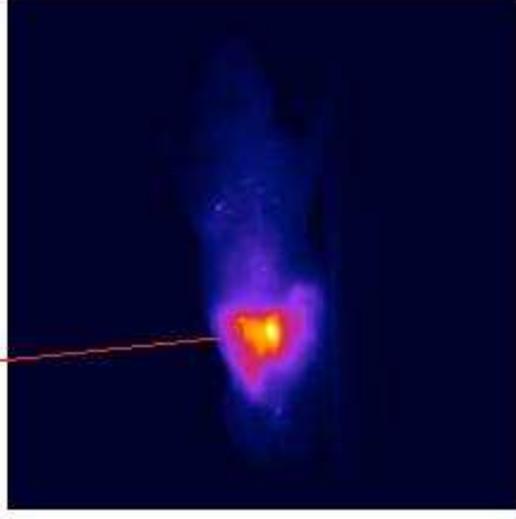
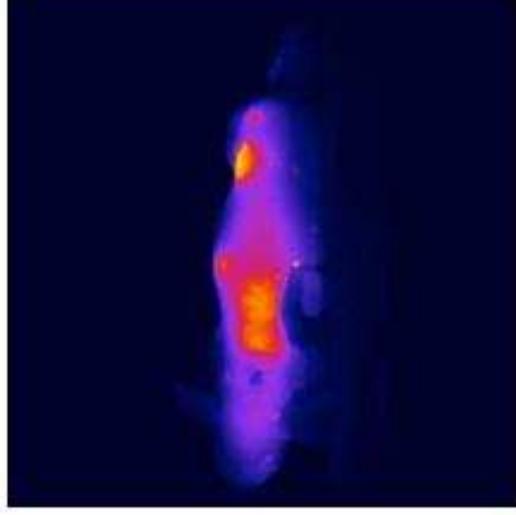
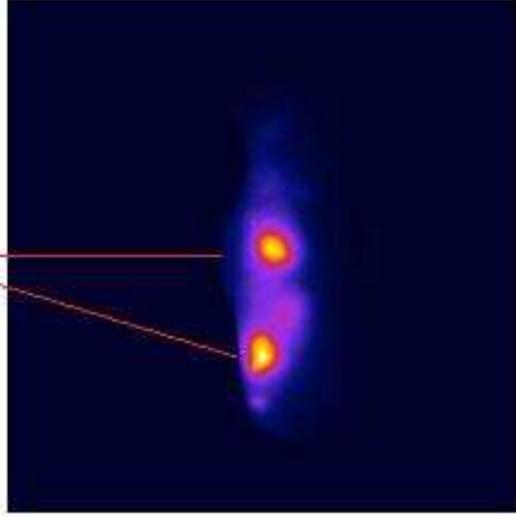
Planar fluorescent images of a mouse

Exposure = 0.1-1 s. Illuminated mouse uniformly with 640 nm.

Superposition of images with and without 665 nm filter.

Injected microspheres under skin near right thigh and chest.

Food present in digestive system



Imaging with ImSpector: 1 mm steps.

- Mouse was illuminated uniformly with 640 nm light.
- Images were taken with and without the 665 nm filter.

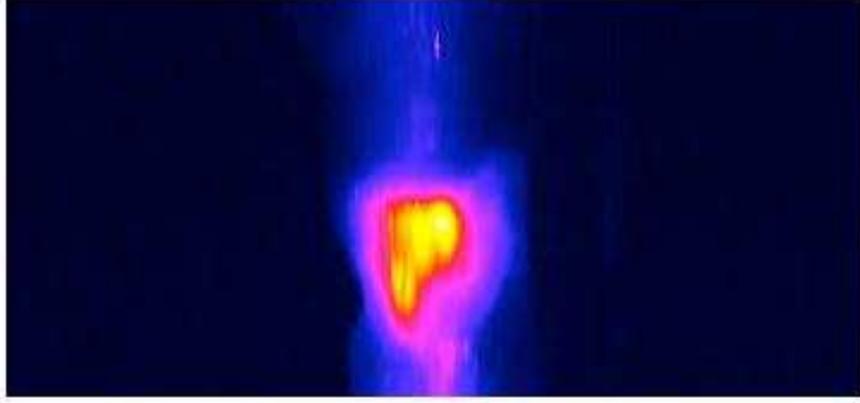
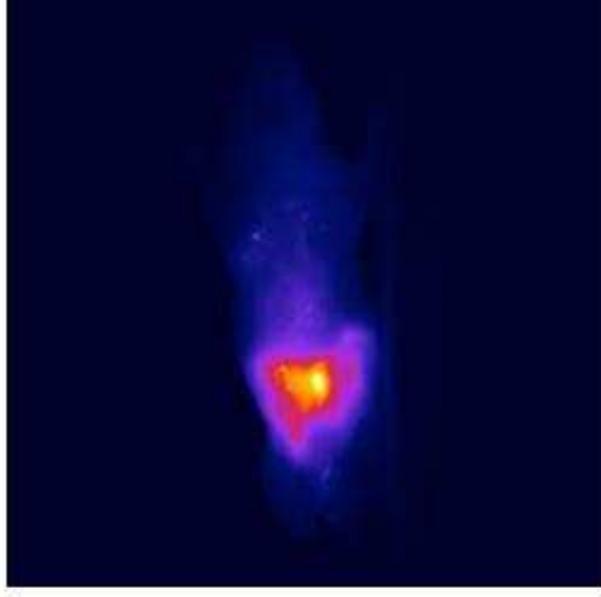
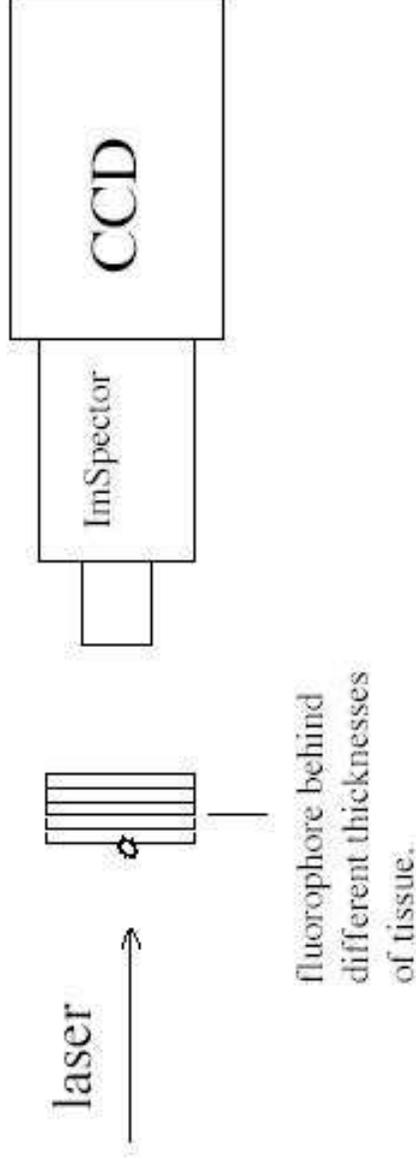


Image with just the CCD



Spectral variations with tissue depth

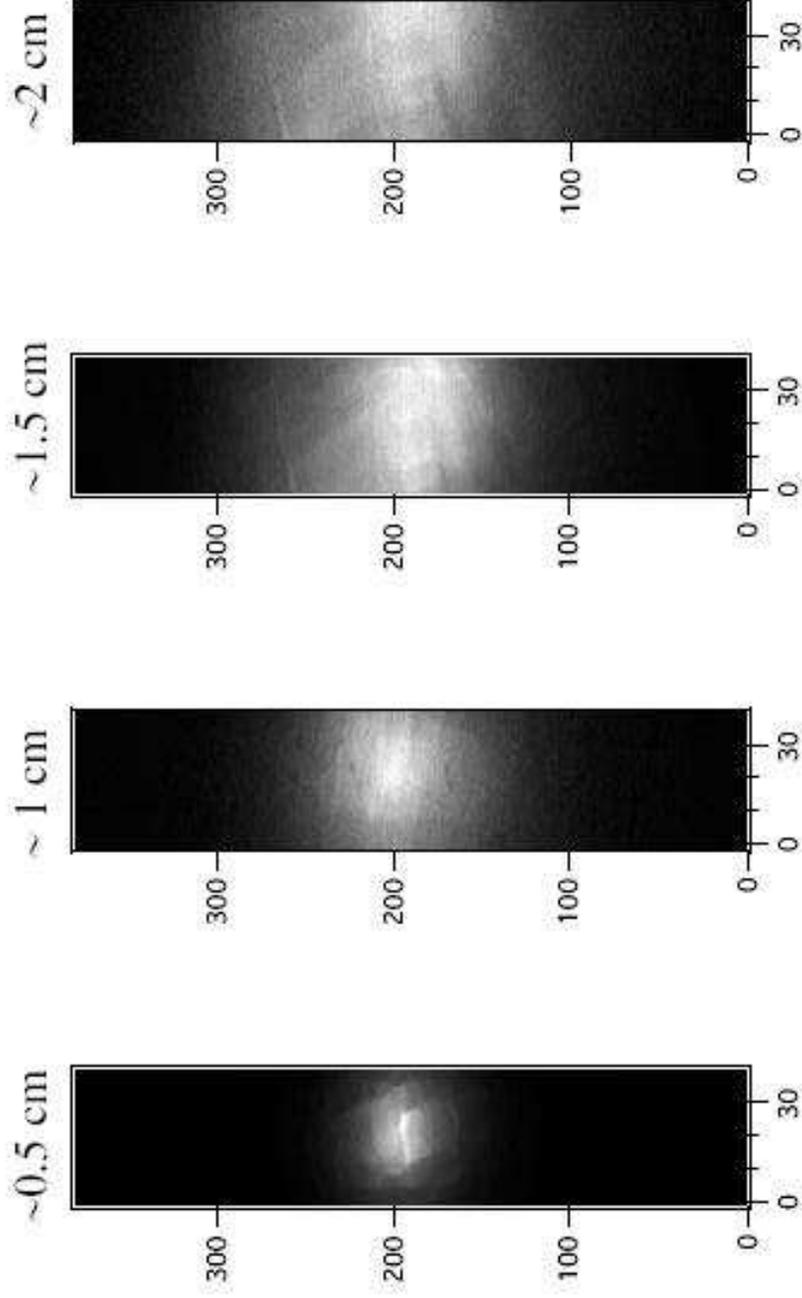
First measurements



- Turkey (2.5 mm slices) and beef (~5 mm slices)
- Up to 5 slices of turkey and 3 slices of beef were used.
- Studied the spectrum through the maximum of the light distribution and at the edge.

Imaging with ImSpector: $250\mu\text{m}$ steps

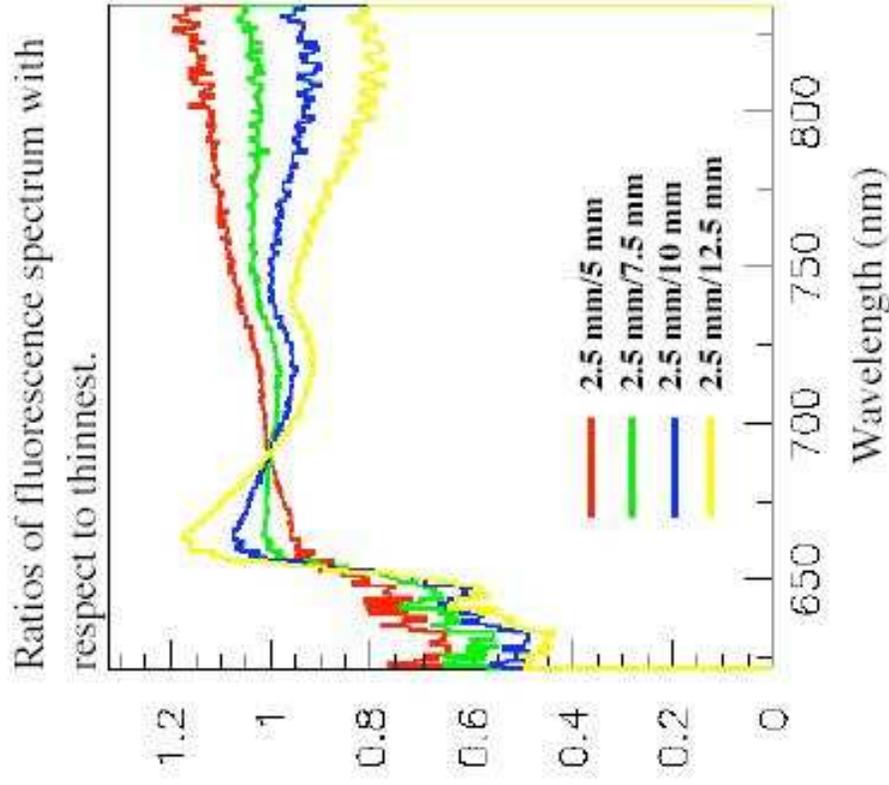
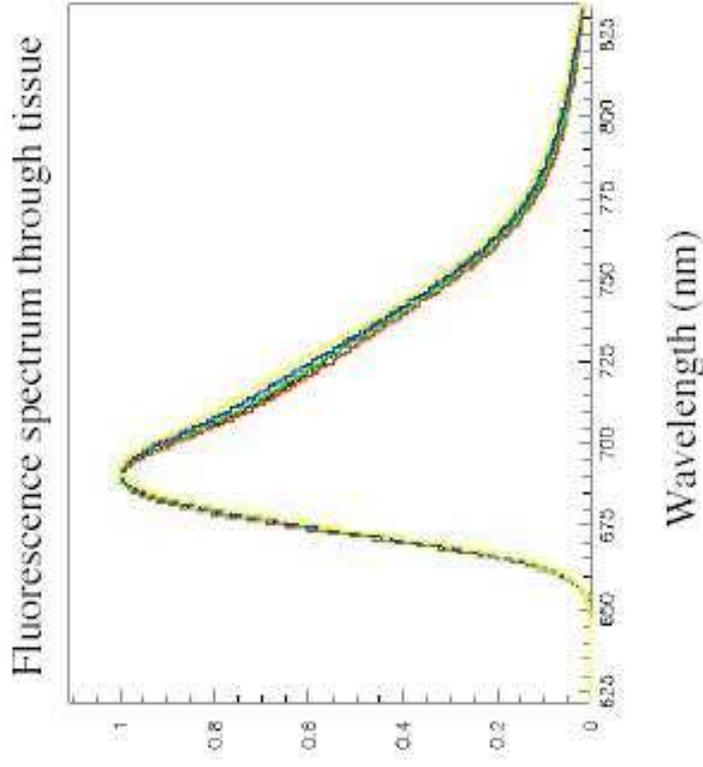
- 4 different thicknesses of beef showing the broadening of the light distribution of



Spectral variations with tissue depth

Turkey meat

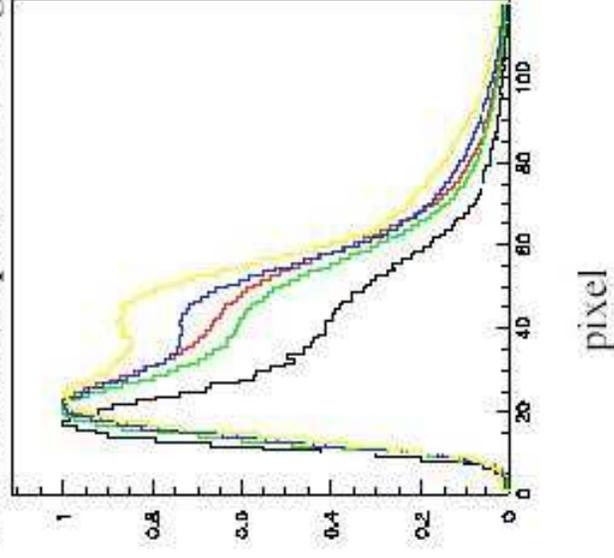
There is a slight variation.



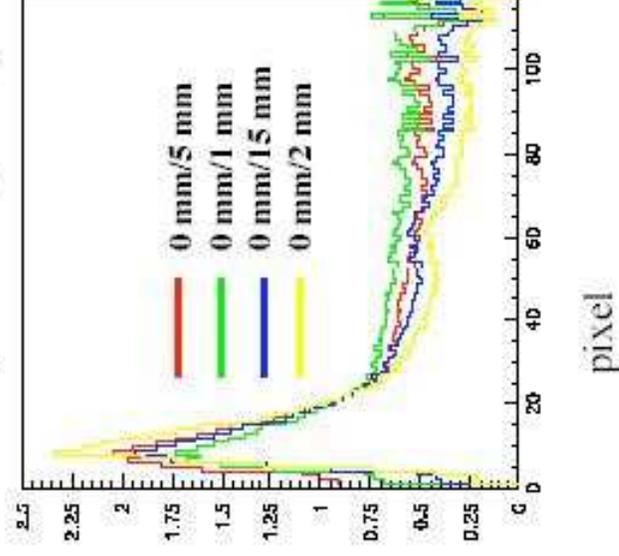
Spectral variations with tissue depth: Beef

There is a significant variation in spectral shape.

Fluorescence spectrum through tissue

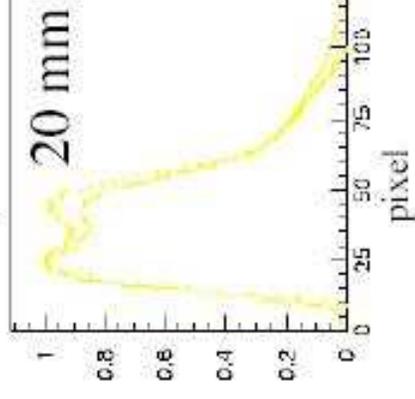
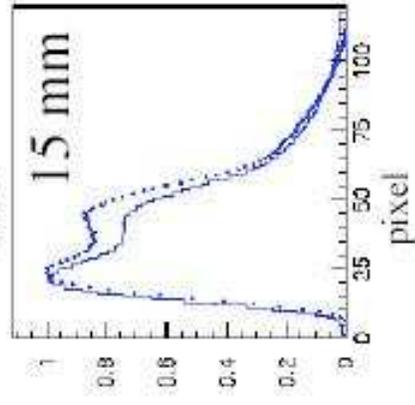
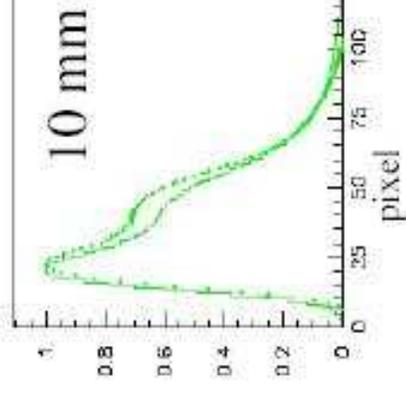
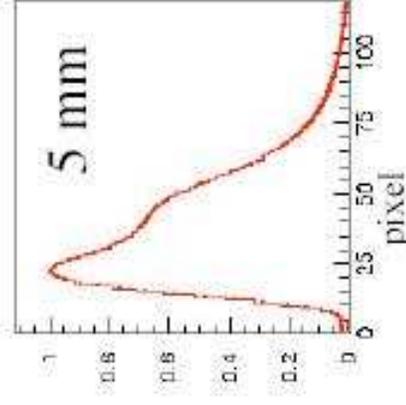


Ratios of fluorescence spectrum with respect to original spectrum.

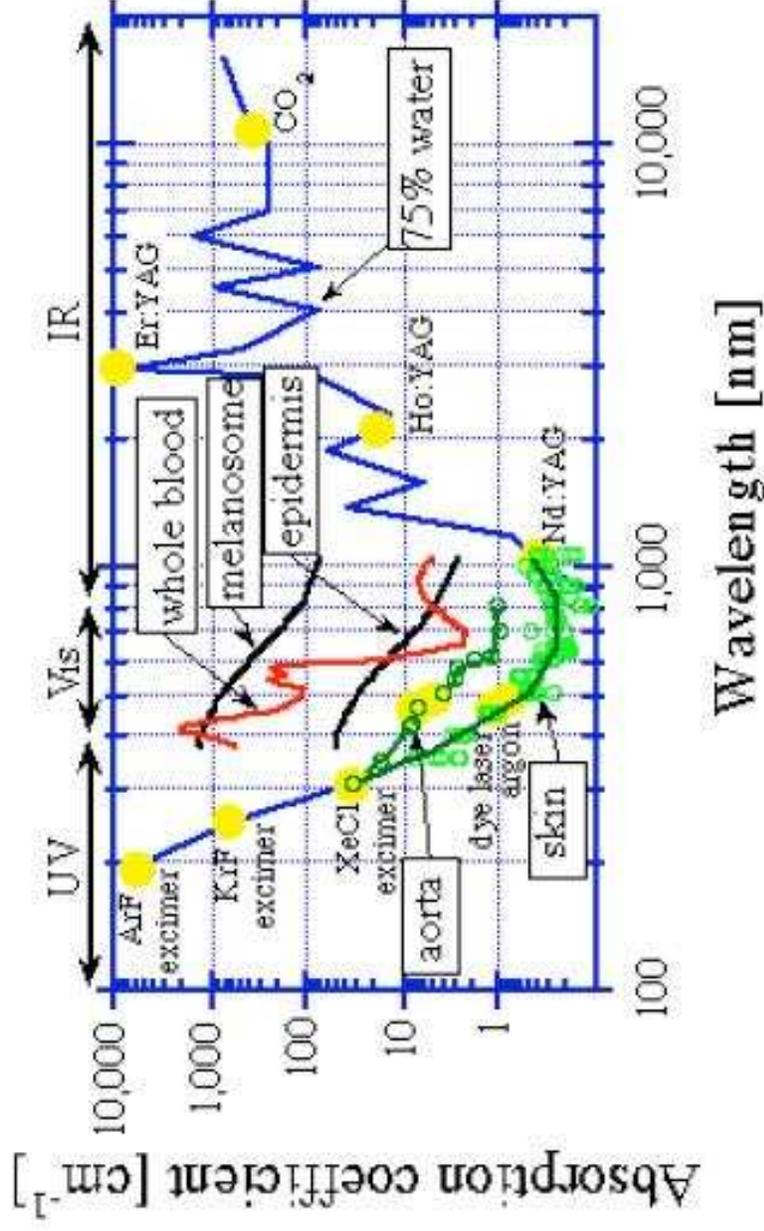


Spectral variation across the light distribution for different thicknesses: Beef

- Dots: edge of distribution;
- Line: center of light distribution



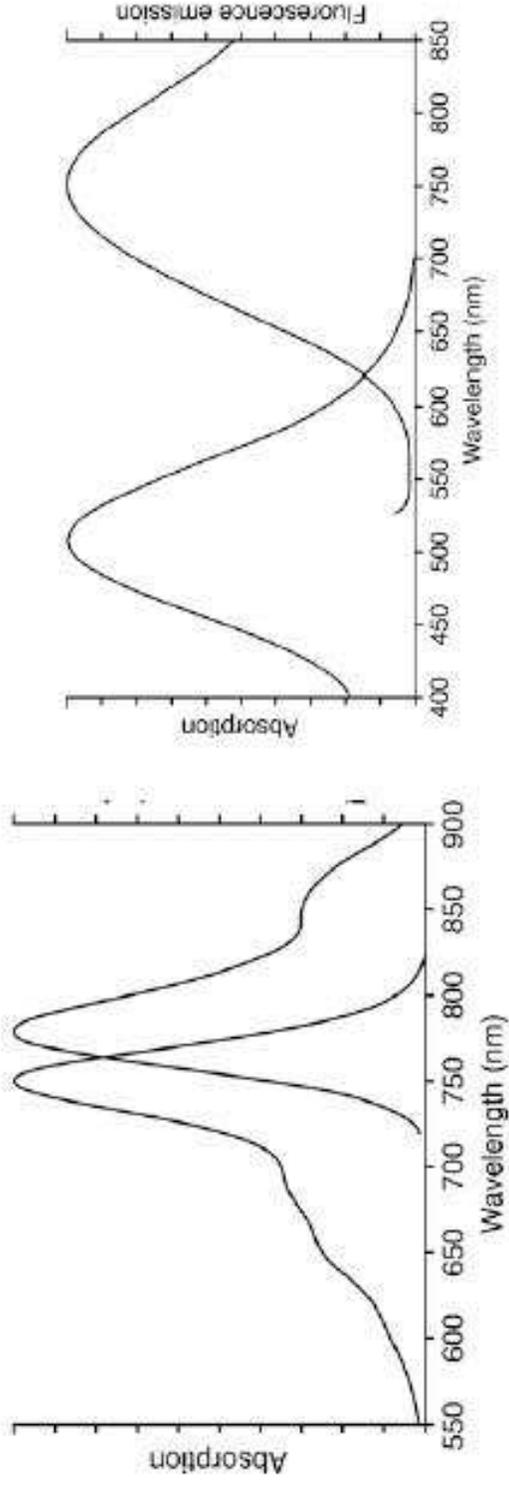
What is happening?



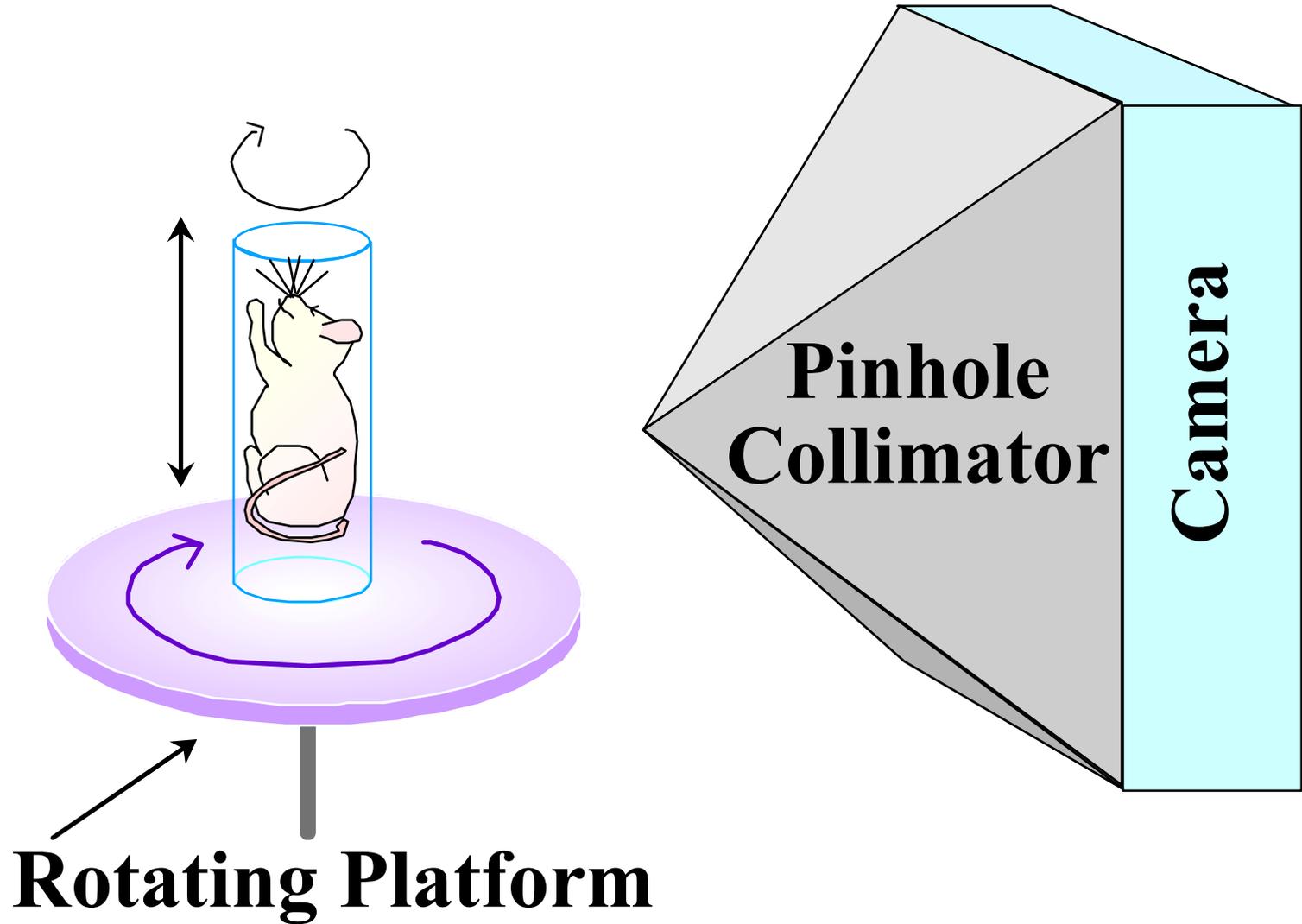
Around 600 - 700 nm we are working near a minimum in absorption

Look for a different fluorophore?

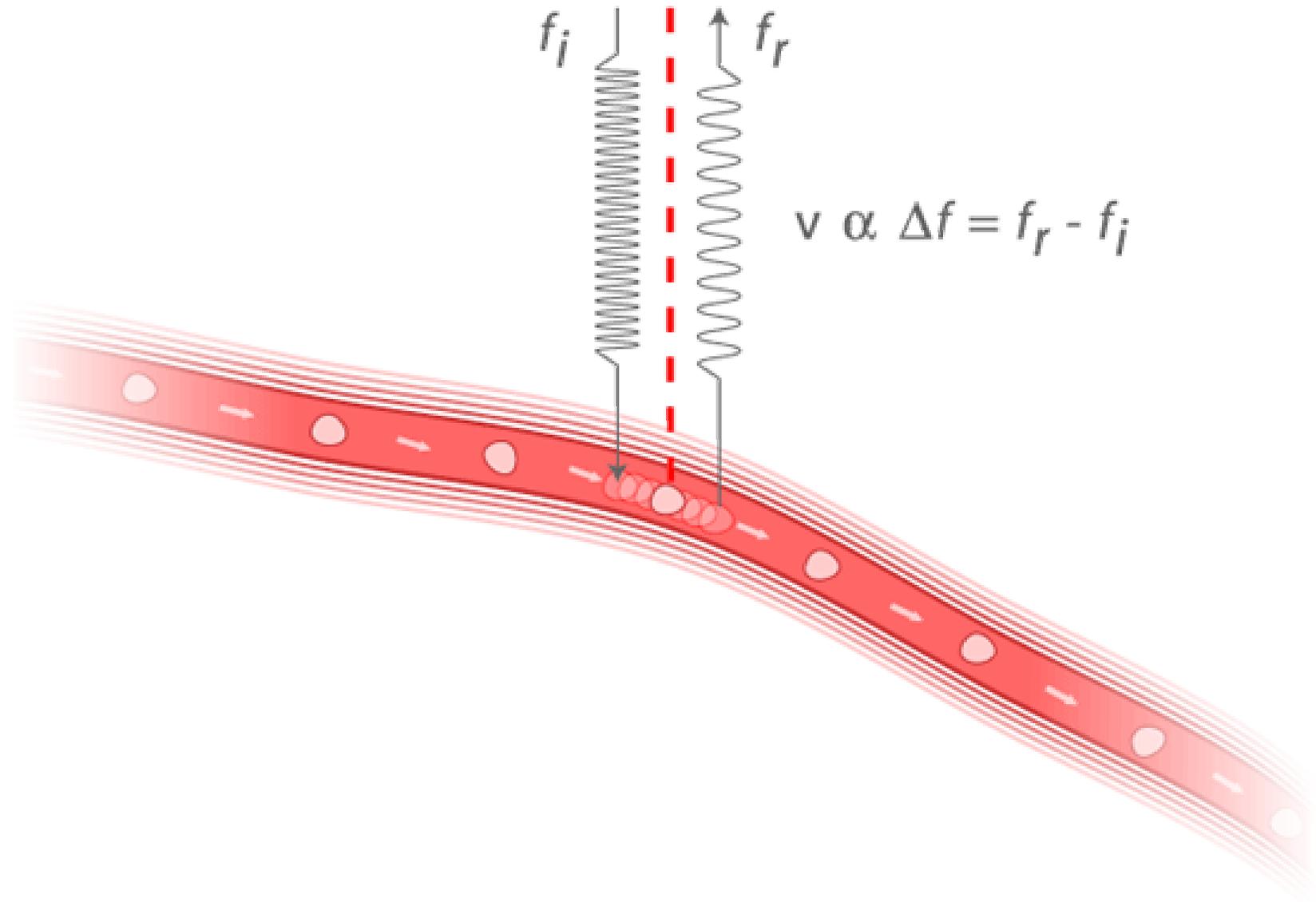
- wider emission to cover wider range of wavelengths
- shift either nearer to the infra red or towards the yellow.



SPECT Camera



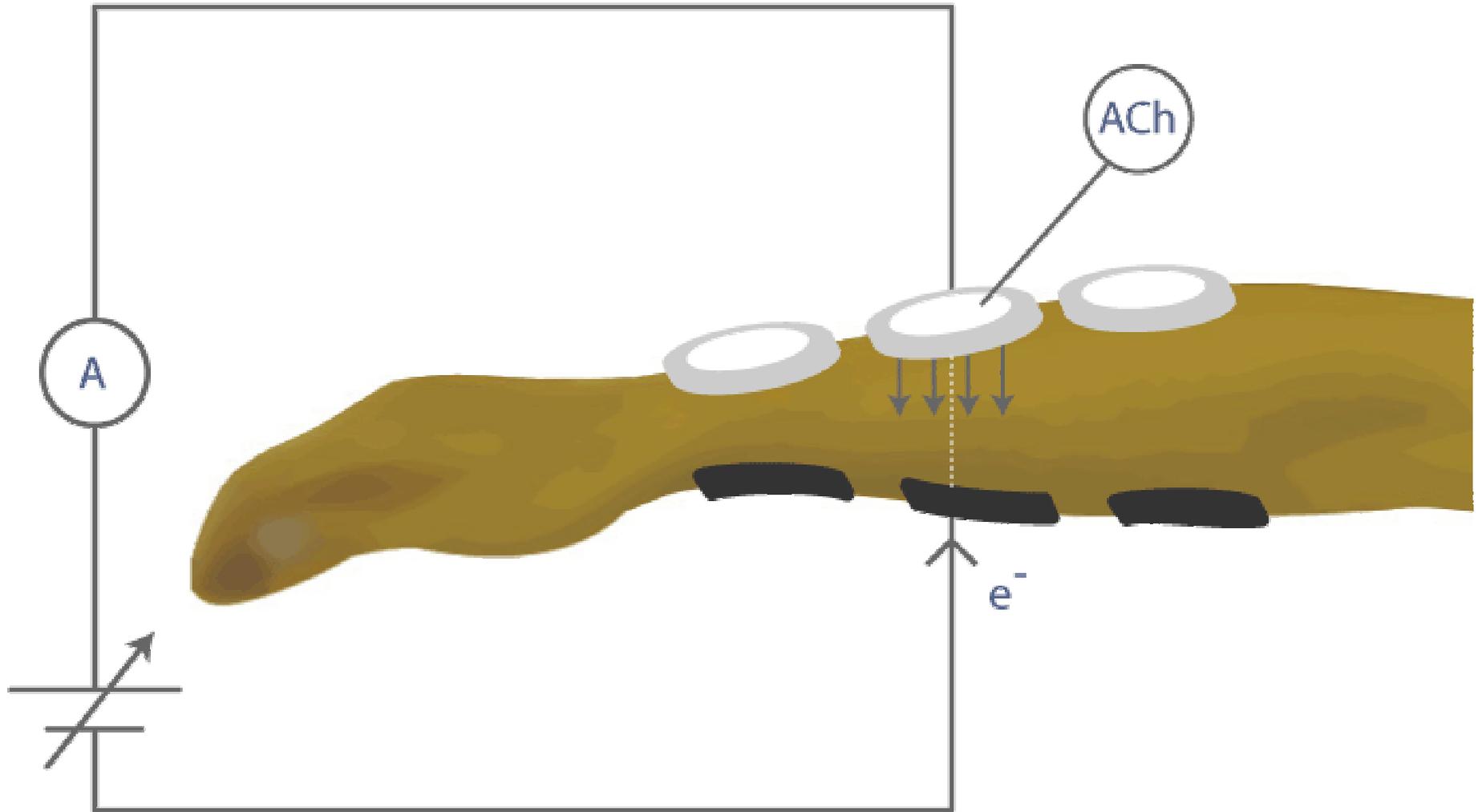
Laser Doppler perfusion imaging



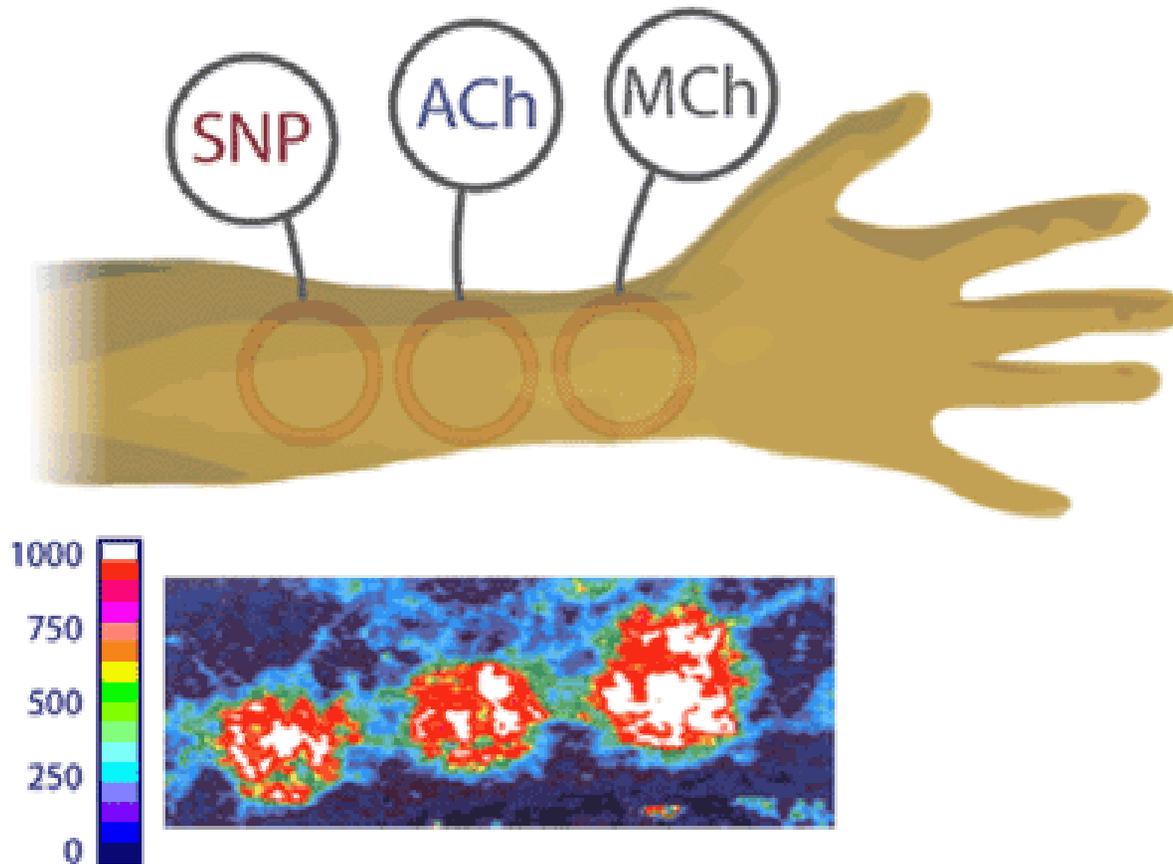
Laser Doppler imaging: quantitative measurement of perfusion



Iontophoresis: quantitative drug delivery

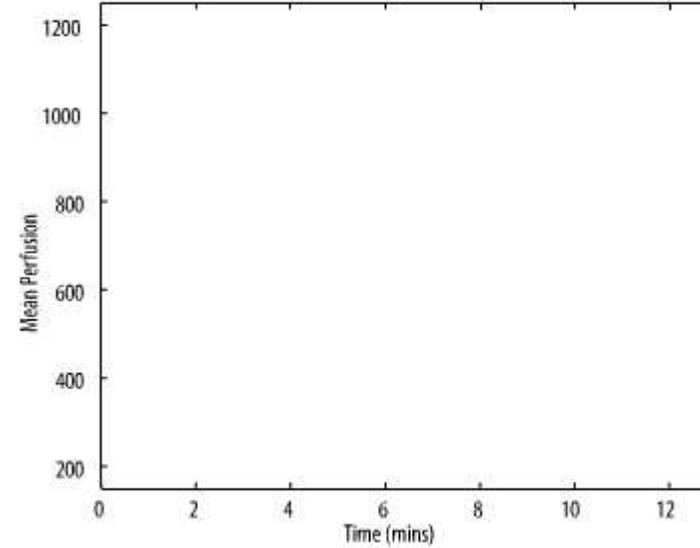
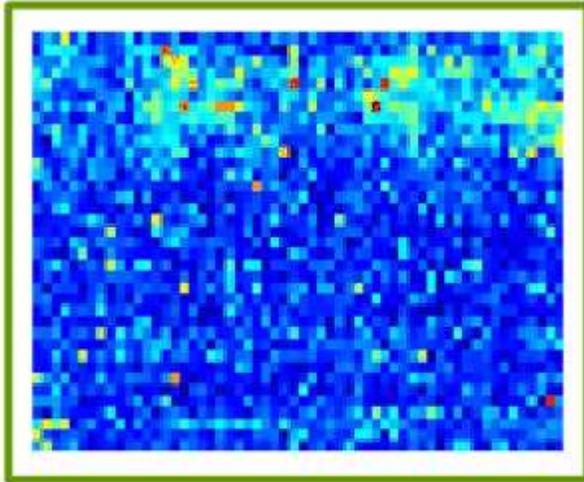


Laser Doppler imaging of perfusion response to iontophoresis

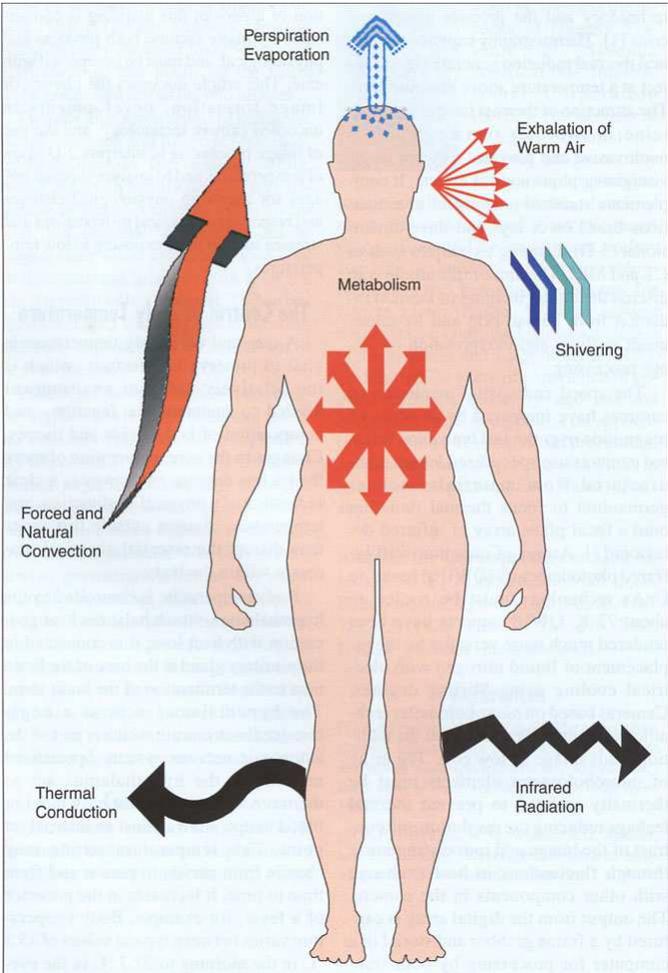


Perfusion response to iontophoresis

Typical response to ACh



Infrared imaging: imaging heat emitted by living organisms





1. A patient with an abnormal asymmetric breast infrared image.

Table 1. Infrared results from normal, cancer, and deceased cancer patients.

Infrared Results	Patients		
	Normal	Cancer	Deceased
Normal	72 72%	35 35%	15 12%
Abnormal	28 28%	65 65%	111 88%

p < 0.0001, chi-square analysis for independence

Asymmetries in breast IR emission seem to indicate and predict breast cancer pathology

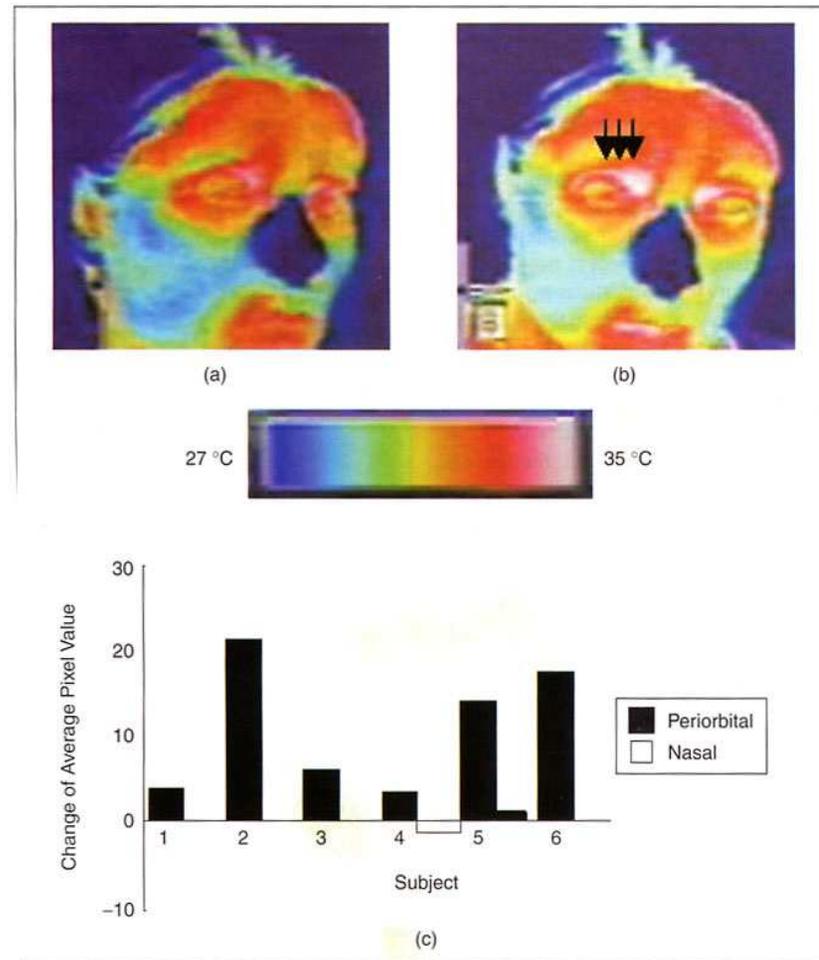


Figure 1. Thermal images of the face for a subject (a) before and (b) 300 ms after an instantaneous startle. Arrows indicate local warming in the periorbital area. The color bar depicts the false coloring scheme from the lowest (27 °C) to the highest (35 °C) temperature. (c) Changes of the average pixel value in the periorbital and nasal areas with auditory startle. The changes are depicted for each subject ($n = 6$ subjects). Positive deviation represents local warming and negative deviation represents cooling.

Infrared imaging shows startle response and can be used to help determine whether a person is lying